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Carotid chemoreceptor control of muscle sympathetic nerve activity in hypobaric hypoxia

Authors: James P Fisher[1], Daniela Flück[2,4], Matthias P Hilty[3] & Carsten Lundby[4,5]

Institutions: [1]School of Sport, Exercise and Rehabilitation Sciences, College of Life and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham, UK; [2]Centre for Heart, Lung and Vascular Health, School of Health and Exercise Sciences, University of British Columbia – Okanagan, Kelowna, British Columbia, Canada; [3]Intensive Care Unit, University Hospital of Zürich, Zürich, Switzerland; [4]Zurich Center for Integrative Human Physiology (ZIHP), Institute of Physiology, University of Zurich, Switzerland; [5]Center for Physical Activity Research (CFAS), University Hospital of Copenhagen, Copenhagen, Denmark.

Running Title: Hypoxia and sympathetic nerve activity

Corresponding author: Dr. James P. Fisher. School of Sport, Exercise and Rehabilitation Sciences, College of Life and Environmental Sciences, University of Birmingham, Edgbaston, Birmingham, UK. Tel: +44 (0)121 414 8011. Fax: +44 (0)121 414 4121. email: j.p.fisher@bham.ac.uk or carsten.lundby@regionh.dk

NEW FINDINGS

What is the central question of this study?

High altitude hypoxia increases muscle sympathetic nerve activity (MSNA), but whether intravenous infusion of dopamine, to blunt the responsiveness of the carotid chemoreceptors, reduces MSNA at high altitude is not known.

What is the main finding and its importance?

MSNA was elevated after 15-17 days of high altitude hypoxia (3,454 m) compared to sea level (432 m) values. However, intravenous dopamine infusion to blunt the responsiveness of the carotid chemoreceptors did not significantly decrease MSNA either at sea level or high altitude, suggesting that high altitude sympathoexcitation arises via a different mechanism.

ABSTRACT

High altitude hypoxia causes pronounced sympathoexcitation but the underlying mechanisms remain unclear. We tested the hypothesis that intravenous infusion of dopamine to attenuate carotid chemoreceptor responsiveness would reduce muscle sympathetic nerve activity (MSNA) at high altitude. Nine healthy individuals (mean [SD]; 26 [4] yr) were studied at sea level (SL, Zurich) and at high altitude (ALT, 3454 m, 15-17 days after arrival), both while breathing the ambient air and during an acute incremental hypoxia test (8 x 3 min stages, $P_{ET}O_2$ 90-45 mmHg). Intravenous infusion of dopamine (3 $\mu g \cdot kg^{-1} \cdot min^{-1}$) and placebo (saline) were administered on both study days, according to a single blind randomized cross-over design. Sojourn to high altitude decreased $P_{ET}O_2$ (to ≈60 mmHg) and increased minute ventilation (V_E; mean±SE; saline [SL, ALT], 8.6±0.5 to 11.3±0.6; dopamine, 8.2±0.5 to 10.6±0.8 $L \cdot min^{-1}$; $P<0.05$) and MSNA burst frequency by ≈80% (saline [SL, ALT], 16±3 to 28±4; dopamine, 16±4 to 31±4 $bursts \cdot min^{-1}$; $P<0.05$) when breathing the ambient air, but were not different with dopamine. Increases in MSNA burst frequency and V_E during the acute incremental hypoxia test were greater at ALT than SL ($P<0.05$). Dopamine did not affect the magnitude of the MSNA burst frequency response to acute incremental hypoxia at either SL or ALT. However, V_E was lower with dopamine than saline administration throughout the acute incremental hypoxia test at ALT. These data indicate that intravenous infusion of low-dose dopamine to blunt the responsiveness of the carotid chemoreceptors does not significantly decrease MSNA at high altitude.

Keywords: autonomic nervous system, high altitude, microneurography

INTRODUCTION

Hypoxia increases the afferent discharge of the carotid chemoreceptors causing reflex increases in ventilatory drive and efferent sympathetic nerve activity directed towards the heart, kidneys and peripheral vasculature (Guyenet, 2000; Kumar & Prabhakar, 2012). In humans, the use of the microneurography technique to directly record sympathetic nerve activity to skeletal muscle vasculature (MSNA) reveals that acute hypoxic exposure elicits variable but typically dose dependent sympathoexcitation once SpO_2 reaches <85% (breathing hypoxic gas mixtures with an of F_iO_2 0.11-0.13%) (Saito *et al.*, 1988; Rowell *et al.*, 1989; Somers *et al.*, 1989; Seals *et al.*, 1991; Duplain *et al.*, 1999). However, such increases in MSNA are dwarfed by those elicited by chronic hypoxic exposure which can reach ≈300% above sea level values, despite reductions in SpO_2 being equivalent (Hansen & Sander, 2003). The mechanism for this difference is unclear, which is unfortunate because similar mechanisms may be important for the pathophysiology of a variety of disease states characterized by chronic sympathoexcitation and chronic intermittent or sustained hypoxaemia (e.g., sleep apnoea related hypertension (Carlson *et al.*, 1993; Narkiewicz & Somers, 1999), chronic obstructive pulmonary disease (Heindl *et al.*, 2001) and chronic heart failure (Leimbach *et al.*, 1986; Narkiewicz *et al.*, 1999)).

Following acclimatization to high altitude there is an augmentation of the ventilatory response to hypoxia that has been ascribed to a sensitization of peripheral chemoreceptors (Forster *et al.*, 1971). Ventilatory and sympathetic chemoreflexes share common afferent pathways and the central neurocircuitry responsible for the efferent activation of the phrenic and sympathetic nerves act in parallel (Guyenet, 2000; Kumar & Prabhakar, 2012). For example, denervation of the carotid body markedly reduces the increases in ventilation and renal sympathetic nerve activity induced by hypoxia in rabbits with pacing-induced congestive heart failure (Marcus *et al.*, 2014). However, it has been suggested that a peripheral chemoreceptor mechanism only modestly contributes to increase in MSNA accompanying chronic exposure to high-altitude hypoxia. Indeed, Hansen and Sander (2003) observed that 100% oxygen breathing following 4 weeks at 5,260 m slightly reduced MSNA (by 7 bursts·min^{-1}), but it still remained robustly elevated (41 bursts·min^{-1}) compared with sea level values (16 bursts·min^{-1}). As acknowledged by the investigators, oxygen administration may have led to a fall in ventilation and an increase in arterial CO_2, which in turn could attenuated the sympathoinhibitory effects of pulmonary stretch reflex engagement and increase central chemoreflex activation. Hyperoxia also has non-specific effects and can cause peripheral vasoconstriction in some individuals (Crawford *et al.*, 1997). Taken together these factors suggest that the contribution of the peripheral chemoreceptors to the control of MSNA in hypoxia warrants further consideration.

Chemoreceptor signalling within the carotid and aortic bodies involves a plethora of excitatory (e.g., adenosine, ATP, acetylcholine and endothelin) and inhibitory neurotransmitters (Lazarov *et al.*, 2009). Dopamine is one of these primary signalling molecules and has an inhibitory effect on high-affinity D_2 autoreceptors (D_2R) located on Type 1 glomus cells (Gonzalez *et al.*, 1994). Intracarotid infusion of dopamine inhibits chemoreceptor afferent activity in dogs (Bisgard *et al.*, 1979), while systemic administration of low-dose dopamine (i.e., <3 μg·kg^{-1}·min^{-1}) is an established method of acutely reducing the responsiveness of the carotid chemoreceptors in humans (Boetger & Ward, 1986; Dahan *et al.*, 1996; Limberg *et al.*, 2016). One study suggests that the suppressive effects of dopamine on the hypoxic ventilatory response are unaltered after individuals have been exposed to isocapnic hypoxia for 8 h (Pedersen *et al.*, 1999). However, ventilatory acclimatization is not complete in humans after 8 h (Dempsey & Forster, 1982) and the effect of low-dose dopamine on the ventilatory response to acute hypoxia following more prolonged high altitude exposure in humans remains unexamined.

The purpose of the present study was to determine whether elevations in steady-state MSNA and ventilation are reduced following 15-17 days of exposure to high altitude hypoxia (3,454 m) (i.e., ambient air breathing) by intravenous infusion of low-dose dopamine (*Aim 1*). We also determined whether the MSNA and ventilatory responses to an acutely administered incremental hypoxia test were attenuated following intravenous dopamine infusion (*Aim 2*) and whether the magnitude of any such inhibitory effect was altered following 15-17 days of exposure to high altitude hypoxia (*Aim 3*). We tested the hypothesis that intravenous dopamine would reduce MSNA and ventilation both at high altitude with ambient air breathing and during an acute incremental hypoxia test, and that the inhibitory effects of dopamine during the incremental hypoxia test would be augmented at high altitude.

METHODS

Ethical Approval.

The experiments were undertaken in accordance with the Declaration of Helsinki, except for registration in a database, and were approved by the Ethical Committee of the Swiss Federal Institute of Technology Zurich (EK 2011-N-51). Written informed consent to take part was obtained from all participants after they had received a detailed verbal and written explanation of the study procedures.

Participant characteristics.

Nine healthy individuals (mean (SD); 26 (4) yr, 179 (9) cm, 75 (10) kg, 1 woman) participated in this study. No participant had a medical history of cardiovascular, respiratory or neurological disease and no participant slept >2,500 m in the 3 months prior to the start of the study. Abstinence from caffeine, alcohol and exercise was requested for the 12 h before experimental sessions.

Experimental measures.

Participants rested in semi-recumbent position while continuous recordings of MSNA, respiratory and cardiovascular variables were made. Heart rate (HR) was monitored using a lead II electrocardiogram (ECG, BioAmp, ADInstruments, Bella Vista, Australia). Mean arterial pressure (MAP) and stroke volume (SV) were recorded on a beat-to-beat basis via finger photoplethysmography (Nexfin, BMEYE B.V, Amsterdam, the Netherlands)(Bogert *et al.*, 2010). Peripheral capillary oxygen saturation (SpO_2) was determined using finger pulse oximetry. However, due to technical issues data steady-state SpO_2 data are presented for n=6 participants and acute incremental hypoxia test SpO_2 data are presented for n=7 participants. Participants breathed through a mouthpiece whilst wearing a nose clip and minute ventilation (V_E), tidal volume (T_V), respiratory frequency (R_f), and the partial pressure of end-tidal oxygen ($P_{ET}O_2$) and carbon dioxide ($P_{ET}CO_2$) were measured breath-by-breath (Cosmed Quark b2, Rome, Italy). Multi-unit recordings of MSNA were obtained (FE185 NeuroAmp EX, ADInstruments, Bella Vista, Australia) from the peroneal nerve using tungsten microelectrodes (FHC, Bowdoin, USA) (Adlan *et al.*, 2017). A reference electrode was inserted subcutaneously 2 to 3 cm away from the recording electrode which was selectively inserted into a sympathetic nerve fascicle. Neural signals were amplified (x100k), filtered (100 Hz high pass, 2,000 Hz low pass), rectified and integrated (absolute value, time constant decay 0.1 s) to obtain a mean voltage sympathetic neurogram. An acceptable MSNA recording exhibited the following characteristics: displayed a pulse-synchronous bursts pattern, had a signal-to-noise ratio of >3:1, was increased during an end-expiratory breath-hold or Valsalva manoeuvre, and was unresponsive to an unexpected loud noise or skin stroking.

Experimental protocol.

Each individual participated in two experimental sessions, the first was conducted in Zurich, Switzerland (SL, 432 m) and the other at the high altitude Jungfraujoch research station (ALT, 3,454 m), 15-17 days after arrival. Participants were familiarized with the study procedures before collection of study data. At both research sites, following instrumentation and acquisition of an acceptable MSNA signal the stability of the recording was verified for ≈10 mins. The experimental protocol then commenced with the collection of 5 min of eupnoea baseline data (SL-baseline, ALT-baseline) (i.e., ambient air breathing). The SL-baseline was then followed by the addition of supplemental CO_2 to the inspired air in order to raise $P_{ET}CO_2$ by 2 mmHg (Altitrainer, SMTEC, Nyon, Switzerland). A 3-min period was permitted to allow a new steady-state to be established (stage 1) following which the incremental hypoxia test commenced (stages 2-8). First, $P_{ET}O_2$ was reduced to 75 mmHg for 3 min, and then incrementally reduced by a further 5 mmHg every 3 min until it reached 45 mmHg, while $P_{ET}CO_2$ remained clamped at +2 mmHg throughout, following the modified methods of Mou *et al.* (1995) (Altitrainer, SMTEC, Nyon, Switzerland). At high-altitude, the ALT-baseline was followed by the addition of supplemental CO_2 and O_2 to the inspired air to raise $P_{ET}O_2$ and $P_{ET}CO_2$ to the SL-baseline levels (stage 1). A 3-min period was permitted to allow a new steady-state to be established following which the incremental hypoxia test (stages 2-8) was repeated using the $P_{ET}O_2$ and $P_{ET}CO_2$ levels observed at SL as a target.

Both at SL and high altitude the protocols described above were repeated during the continuous infusion of dopamine into the antebrachial vein at a rate of 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ in accordance with several previous studies in humans (Boetger & Ward, 1986; Dahan *et al.*, 1996; Limberg *et al.*, 2016). Dopamine infusion was commenced a minimum of 10 minutes prior to any data collection. Termination criteria for dopamine infusions were: signs of poor perfusion (cyanosis or pallor), technical difficulties in monitoring ECG or systolic blood pressure, subject's desire to stop, ST elevation (≥ 1.0 mm, in leads other than V1 or aVR), sustained ventricular tachycardia, arrhythmias other than sustained ventricular tachycardia (including multifocal premature ventricular complexes, triplets of premature ventricular complexes, supraventricular tachycardia, heart block, or bradyarrhythmias), chest pain, systolic blood pressure > 250 mmHg. Termination criteria were not met on any occasion.

Data analysis.

Data was acquired using the Powerlab 16/35 data acquisition system and Labchart Pro software (ADInstruments, Bella Vista, Australia). ECG, MAP, SV, and SpO2 were sampled at 1,000 Hz and raw MSNA was sampled at 20,000 Hz and stored for offline analysis (LabChart 7 Pro v7.3.5 and Powerlab, ADInstruments, Bella Vista, NSW, Australia). Cardiac output (CO) was calculated as SV x HR, and total peripheral resistance (TPR) as MAP / CO. Sympathetic bursts were identified by a single observer (JPF) using a semi-automated scoring system created using Spike 2 (Cambridge Electronic Design, Cambridge, UK). MSNA was characterised in terms of burst incidence (bursts·100 heartbeats^{-1}) and burst frequency (bursts·min^{-1}). In one individual microneurography was unsuccessful, and in another individual the MSNA recording was lost during the final stages of the acute incremental hypoxia test. As a consequence, the steady-state MSNA data are presented for n=8 participants and acute incremental hypoxia test MSNA data are presented for n=7 participants.

Statistics.

Statistical analysis was performed using SPSS software, version 19 (SPSS Inc, Chicago, Ilinois). Physiological data were statistically analyzed using repeated measures analysis of variance (ANOVA), with Greenhouse-Geisser corrections applied where significant violations of the sphericity assumption were detected. More specifically, to determine whether dopamine lowers steady-state MSNA and ventilation at high altitude (SL-baseline vs. ALT-baseline; Aim 1) a two-way repeated measures ANOVA was used, in which the factors were altitude (SL vs. ALT) and infusion (saline vs. dopamine), as well as the interaction between them. To determine whether dopamine lowers MSNA and ventilation during an acutely administered hypoxic test

(Aim 2), and whether the magnitude of this inhibitory test is augmented at high altitude (Aim 3), this model was extended to a three-way repeated measures ANOVA, additionally including the incremental hypoxia test stage (stages 1-8), as well as all two- and three-way interactions. Where the three-way interaction (altitude x infusion x stage) was not found to be significant, the approach was simplified by dividing the analysis into separate models for each altitude, each containing the infusion, hypoxia test stage and an interaction as factors. Post hoc analysis was employed using Student's t tests with Bonferroni correction to investigate significant main effects and interactions. Data expressed as mean (standard deviation) unless otherwise stated. $P<0.05$ was considered statistically significant.

RESULTS

High altitude hypoxia, ventilation and MSNA with ambient air breathing.

Sojourn to high altitude decreased $P_{ET}O_2$ (saline [SL, ALT], 93 (2) to 60 (4); dopamine [SL, ALT], 90 (5) to 57 (2) mmHg. P<0.001), $P_{ET}CO_2$ (saline [SL, ALT], 40 (2) to 31 (1); dopamine [SL, ALT], 41 (3) to 32 (2) mmHg. P<0.001) and SpO_2 (saline [SL, ALT], 97 (1) to 92 (2); dopamine [SL, ALT], 97 (1) to 89 (2) %. P<0.001) and increased V_E (by ≈2.5 $L \cdot min^{-1}$, P<0.002. Figure 1.) With dopamine, $P_{ET}O_2$ was slightly lower (P=0.023) and $P_{ET}CO_2$ slightly higher (P=0.003) compared to saline, but no altitude x infusion interaction was observed. SpO_2 was not different with dopamine at SL (P=0.789), whereas it was lower with dopamine at ALT (P=0.028). V_E was not different with dopamine (P=0.186), and no altitude x infusion interaction was noted for any respiratory variable (Figure 1).

ALT increased MSNA burst frequency (by ≈80 %, P=0.019), MAP (by ≈12 %, P=0.002) and HR, while MSNA burst incidence (saline [SL, ALT], 25±16) to 38±11); dopamine [SL, ALT], 26 (21) to 40 (12) $bursts \cdot 100\ heartbeats^{-1}$. P=0.088) tended to increase (Figures 1 and 2). However, CO (P<0.646), SV and TPR (P<0.100), were not different at ALT (Figure 3). Dopamine infusion increased HR (P=0.001) and CO (P<0.001), decreased TPR (P=0.035), but had no effect on MSNA burst frequency (P=0.289), MSNA burst incidence (P=0.555), MAP (P=0.837) and SV (P=0.119). No altitude x infusion interaction was noted for any MSNA or cardiorespiratory variable.

Acute incremental hypoxia at SL and ALT: ventilation and MSNA.

During the acute incremental hypoxia test, $P_{ET}O_2$ and SpO_2 were decreased (P<0.001) in the same stepwise manner under all conditions (Table 1, 2 and 3). At SL, $P_{ET}CO_2$ remained stable throughout the incremental hypoxia test (P=0.177) and there were no differences between the saline and dopamine conditions (P=0.523). $P_{ET}CO_2$ was ≈3 mmHg lower (P<0.001) at ALT than at SL during the test, and although no differences were observed between the saline and dopamine conditions (P=0.177), $P_{ET}CO_2$ fell during stages 3 and 4 (P<0.05 vs. stage 1).

V_E, T_V, and R_f increased (P<0.001) with acute incremental hypoxia at both SL and ALT, but the magnitude of this increase was greater at altitude (P<0.001. Figure 4, Tables 2 and 3). At SL, dopamine did not affect the increase in V_E (P=0.298), T_V (P=0.120), and R_f (0.922) with incremental hypoxia, however at ALT V_E (P=0.023), T_V (P=0.047), and R_f (P=0.050) were lower with dopamine. For V_E, T_V, and R_f, no interactions were noted between infusion and incremental hypoxia test stage for either the SL or ALT conditions.

MSNA burst frequency increased similarly during the acute incremental hypoxia test at SL (P=0.028) and ALT (P=0.023) (Figures 5 and 6, Tables 2 and 3). MSNA burst frequency was higher during the incremental hypoxia test with dopamine at both SL (P=0.051) and ALT (P=0.015). MAP and CO increased progressively during the incremental hypoxia test at both SL and ALT (P<0.01), but the magnitude of this increase was greater at altitude (P<0.001). Dopamine did not affect MAP at either SL (P=0.590) or ALT (P=0.308), but it did increase CO at SL (P=0.041). TPR was progressively decreased (P<0.001) with acute incremental hypoxia both at SL and ALT. For MSNA burst frequency, MAP, CO and TPR no interactions were noted between infusion and incremental hypoxia test stage for either the SL or ALT conditions.

DISCUSSION

We sought to ascertain whether the sympathoexcitation and hyperventilation associated with hypoxia are lowered at high altitude by the intravenous infusion of low-dose dopamine to attenuate carotid chemoreceptor responsiveness. The major novel finding of the present study are; 1) the elevations in MSNA and ventilation observed after 15-17 days of high altitude hypoxia (3,454 m) were not reduced by intravenous dopamine infusion when participants were breathing ambient air, 2) the magnitude of the increase in MSNA during an acute incremental hypoxia test performed at sea level and high altitude was not affected by dopamine, and 3) ventilation was elevated during acute incremental hypoxia at high altitude compared to sea level, but was lower at high altitude with dopamine. In the following paragraphs a context will be provided to these findings in light of the relevant literature and several important methodological considerations relating to our experimental design will be discussed.

MSNA, hypoxia and dopamine

The carotid chemoreceptors are classically recognized for their oxygen sensing function and consummate reflex increase in ventilation upon activation, however they also possess important autonomic cardiovascular effects with relevance for health and disease (Guyenet, 2000; Kumar & Prabhakar, 2012). Acute hypoxia increases the afferent discharge of the carotid chemoreceptors causing an increase sympathetic nerve activity to several regions (Guyenet, 2000; Kumar & Prabhakar, 2012). However, the contribution of the carotid chemoreceptors to the sympathoexcitatory effects of chronic hypoxia is more controversial. Indeed, in the present study sojourn to 3,454 m for 15-17 days markedly increased steady-state MSNA, however this was not attenuated with dopamine administration. This supports the findings of Hansen and

Sander (2003) who observed that 100% oxygen breathing after 4 weeks at 5,260 m only minimally reduced MSNA (from 48 to 41 bursts·min^{-1}). What is more, we observed MSNA responses to acute incremental hypoxia at altitude were also unaltered with intravenous dopamine infusion. At present the mechanisms underlying such high altitude sympathetic hyperactivity remain obscure and no satisfactory explanation exists. Hansen and Sander (2003) furthermore demonstrated that cardiopulmonary baroreceptor loading at altitude only has a minor effect on MSNA. Remaining possibilities include central changes in the long-term potentiation of sympathetic outflow (Xie *et al.*, 2001), attenuated central sympathoinhibitory pathways such as nitric oxide (Ogawa *et al.*, 1995) and alterations in other reflex control mechanisms.

Ventilation, hypoxia and dopamine

D_2-receptor blockade in rats and cats increases carotid chemoreceptor afferent activity and ventilation (Tatsumi *et al.*, 1995; Huey *et al.*, 2003). Moreover, in the same species, 24-48 h of chronic hypoxia decreased carotid body dopaminergic inhibition (Tatsumi *et al.*, 1995; Huey *et al.*, 2003). Domperidone infusion to block D_2-receptors similarly augmented the hypoxic ventilatory response before and after 4 h of isocapnic hypoxia in goats (Janssen *et al.*, 1998) and 8 h of isocapnic hypoxia in humans (Pedersen *et al.*, 1999), suggesting that dopaminergic inhibitory mechanisms are preserved. It has been suggested that the magnitude of the reduction in chemosensitivity with dopamine is reflective of the baseline chemosensitivity, and thus the endogenous dopamine concentration (Ward, 1984). As such, our finding that ventilation was lower with dopamine compared to saline administration during an acute hypoxia test following 15-17 days at high altitude could suggest that endogenous dopamine levels at the carotid chemoreceptor are decreased at altitude in humans. However, as we did not administer a D_2-

receptor blockade (e.g., domperidone) we cannot provide a definitive insight into this issue. Our findings are however compatible with the view that dopamine is an important inhibitory neurotransmitter in the human carotid body and the inhibitory effects of its endogenous provision evoke a more pronounced effect on ventilation during an acute hypoxia test following 15-17 days of high altitude exposure compared to that observed at sea level. The differential effects of dopamine on the ventilatory and MSNA responses described may be attributable to the actions of distinct populations of glomus cells (Paton *et al.*, 2013).

Experimental considerations

The results and conclusions of the present study must be viewed in light of several experimental considerations. Contrary to previous reports (Welsh *et al.*, 1978; Pedersen *et al.*, 1999), low-dose dopamine was not found to suppress the ventilation under conditions of normoxia and acute hypoxia at SL (P=0.186), however $P_{ET}CO_2$ was increased and $P_{ET}O_2$ was decreased with dopamine, consistent with a mild ventilatory suppression (Welsh *et al.*, 1978). The differences between studies may be attributable to the marked inter-individual differences in the ventilatory response to dopamine *per se* (Limberg *et al.*, 2016). In a recent report, 30% of individuals were shown to have an increase rather than a decrease in the ventilatory response to acute hypoxia with dopamine infusion at 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (Limberg *et al.*, 2016). Differences in the administration of hypoxia and the analytical approaches used to assess the physiological effects of hypoxia, also makes it challenging to directly compare studies employing low-dose dopamine to inhibit the chemoreflex. We utilized an acute incremental hypoxia test that was administered in the form of sequential stepwise reductions in the target $P_{ET}O_2$, following a modification of the methods of Mou *et al.* (1995). An alternative approach would have been to employ short discrete discontinuous bouts of hypoxia, either in a repeated or stepwise manner. This would have perhaps better circumvented issues associated with potential carry-over effects between the stages of hypoxia and any hypoxic ventilatory depression (Teppema & Dahan, 2010). It is also acknowledged that the hypoxic ventilatory response in humans can be expressed relative to SpO_2, but due to technical issues this data was not acquired in all participants. Nevertheless, the approach we employed enabled to consistently control the stepwise reductions to the target $P_{ET}O_2$ under all conditions.

High doses of dopamine (i.e., >3 $\mu g \cdot kg^{-1} \cdot min^{-1}$) may activate α− and β-adrenoreceptors with well-defined cardiovascular actions and can result in hypertension (Stickland *et al.*, 2011). At a low-dose (<3 $\mu g \cdot kg^{-1} \cdot min^{-1}$), dopamine infusion can however cause vasodilatation and increased blood flow through several regions by activation of postsynaptic D_1-receptors in coronary, renal, mesenteric and cerebral circulations and presynaptic D_2-receptors in the peripheral and kidney vasculature (Clark & Menninger, 1980). As mentioned above, the peripheral chemoreceptors also exert effects on reflex cardiovascular control (Guyenet, 2000; Kumar & Prabhakar, 2012). In agreement with other studies (Eugene, 2016; Limberg *et al.*, 2016), low-dose dopamine infusion decreased TPR in the present study. Such vasodilatory actions of dopamine likely contributed to the elevation of HR and CO under steady-state conditions and during the acute incremental hypoxia test, and the elevated MSNA burst frequency during the acute incremental hypoxia test. This likely occurred via baroreflex mechanism in order to preserve MAP, which was largely unchanged. It is acknowledged that the occurrence of such secondary compensatory hemodynamic adjustments arguably constrains the interpretation of the data generated. In addition, dopamine has been shown to have direct cardiac effects (Holmes & Fowler, 1962), which may have contributed to the elevated HR and CO observed with dopamine infusion. The systemic administration of low-dose dopamine (i.e., 3 $\mu g \cdot kg^{-1} \cdot min^{-1}$) was undertaken in accordance with several previous studies in humans (Boetger & Ward, 1986; Dahan *et al.*, 1996; Limberg *et al.*, 2016). However, it is important to note that despite a change in the prevailing MSNA with dopamine, the responses to the acute incremental hypoxia test were unchanged (i.e., no infusion x stage interaction, noted either at SL or ALT). An alternative approach would have been to administer dopamine directly into the carotid artery and/or record carotid chemoreceptor afferent nerve discharge to verify carotid body inhibition, as has been performed in dogs (Bisgard *et al.*, 1979; Stickland *et al.*, 2007), but this extremely invasive technique was unfeasible. The hypoxic pressor response was augmented at altitude, but rather than occurring via a sympathetic vasoconstrictor effect, appeared to occur secondary to an augmented increase in CO. Whether this relates to a difference in autonomic cardiac control relating to chemoreflex activation *per se* warrants further investigation.

We attempted to control $P_{ET}CO_2$ such that it remained at SL isocapnic conditions throughout the acute incremental hypoxia test, however it was lower (≈3 mmHg) at altitude. Therefore, it is possible that the sympathoexcitatory and hyperventilatory responses to the test were underestimated at ALT compared to SL. However, no differences in $P_{ET}CO_2$were noted between the saline and dopamine conditions. Ventilation was higher at high altitude when participants were breathing air with $P_{ET}CO_2$ and $P_{ET}O_2$ maintained at sea level values (Figure 4A) compared to when they were breathing the ambient air (i.e., poikilocapnic hypoxia). A potential explanation for this is that the supplemental CO_2 provided to the inspired air to return it to sea level values stimulated the chemoreceptors at high-altitude (e.g., due to central acid-base balance alterations) (Ainslie *et al.*, 2013). We observed subtle differences in the MSNA responses to altitude and acute incremental hypoxia, when expressed as burst frequency (bursts·min^{-1}) or burst incidence (bursts·100 heartbeats^{-1}). For example, steady-state MSNA frequency and burst incidence were both robustly elevated at altitude, but likely due to a concomitantly elevated (P=0.088) HR. When interpreting sympathetic effects of altitude and dopamine in the present study we have principally relied upon burst frequency data (bursts per unit time). SV and CO were monitored using finger photoplethysmography, and although this approach can reliably track changes in these parameters during laboratory-based manoeuvres (Bogert *et al.*, 2010), the indirect nature of this method is a potential limitation. Finally, the small sample size is a potential limitation of our study. Although the number of participants is similar to earlier work employing a within subject design to examine the influence of high altitude on MSNA (Hansen & Sander, 2003), we acknowledge the potential for a type II error to have occurred.

In this study, we examined the effects of intravenous low-dose dopamine on neural cardiovascular control following chronic hypobaric hypoxia (15-17 days at 3,454 m). Intravenous dopamine infusion did not lower the increases in MSNA at high altitude when ambient air was breathed, furthermore the MSNA response to an acute incremental hypoxia test was not affected by dopamine infusion either at sea level and high altitude. These findings support the view that intravenous low-dose dopamine to attenuate the responsiveness of the carotid chemoreceptors does not diminish the sympathoexcitation of high altitude, but should be viewed in light of the methodological considerations relating to our experimental design that are discussed above.

CONFLICTS OF INTERESTS/COMPETING INTERESTS

The authors have no conflicts of interest/competing interests.

AUTHOR CONTRIBUTIONS

JPF was involved with the conception and design of the experiments, the collection, analysis and interpretation of data, and drafting the first version of the article. DF and MPH were involved in collection, analysis and interpretation of data, and revising the article critically for important intellectual content. CL was involved in the conception and design of the experiments, collection and interpretation of data, and revising the article critically for important intellectual content. All authors have approved the final manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriate. All persons designated as authors qualify for authorship and all those who qualify for authorship are listed.

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Table 1. Selected cardiorespiratory responses to the acute incremental hypoxia test at Zurich (SL, 408 m) and Jungfraujoch research station (ALT, 3,454 m) during infusion of saline or dopamine.

	Stage of incremental hypoxia test							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
$P_{ET}O_2$ (mmHg)								
SL saline	96.7 (2.5)	74.5 (1.4)	69.9 (1.5)	64.8 (0.7)	59.0 (1.4)	55.0 (0.9)	49.8 (0.7)	45.0 (1.3)
SL dopamine	95.4 (4.4)	73.6 (1.0)	70.9 (1.8)	64.9 (1.6)	60.0 (1.4)	54.9 (0.8)	49.9 (1.5)	45.5 (1.0)
ALT saline	98.7 (12.7)	79.2 (10.8)	70.1 (0.9)	63.9 (1.7)	59.7 (0.6)	54.6 (2.2)	50.1 (0.8)	44.7 (0.6)
ALT dopamine	95.5 (4.6)	75.7 (1.5)	69.1 (1.2)	64.7 (1.8)	59.5 (1.0)	55.1 (0.7)	50.0 (1.2)	44.7 (0.6)
$P_{ET}CO_2$ (mmHg)								
SL saline	41.5 (2.1)	41.7 (2.1)	41.7 (2.0)	41.5 (2.0)	41.7 (2.3)	41.7 (2.2)	41.6 (2.2)	41.6 (2.2)
SL dopamine	42.9 (2.9)	42.9 (2.6)	43.1 (2.8)	43.2 (3.0)	43.3 (3.0)	43.1 (3.1)	43.0 (2.9)	43.0 (3.1)
ALT saline	39.5 (1.8)	37.8 (2.8)	35.3 (2.7)	35.3 (2.1)	38.8 (1.9)	39.9 (1.8)	40.2 (1.6)	39.9 (1.1)
ALT dopamine	39.7 (2.1)	39.4 (2.1)	36.0 (2.6)	36.1 (2.5)	37.7 (2.2)	40.5 (1.8)	40.5 (1.8)	40.6 (2.1)
SpO_2 (%)								
SL saline	98.0 (0.8)	96.2 (0.9)	95.5 (0.9)	94.6 (1.0)	93.0 (1.2)	91.3 (1.8)	88.0 (2.5)	83.6 (3.7)

SL dopamine	97.9 (1.2)	95.8 (1.1)	95.6 (1.2)	94.2 (1.5)	92.9 (2.1)	90.7 (2.5)	87.6 (2.6)	84.1 (3.0)
ALT saline	98.5 (0.9)	96.7 (1.4)	95.7 (0.8)	94.4 (1.1)	92.8 (1.5)	90.2 (1.9)	87.2 (2.7)	81.7 (3.5)
ALT dopamine	97.9 (1.0)	95.9 (1.2)	95.1 (1.2)	93.7 (1.2)	92.3 (1.3)	90.0 (1.2)	87.5 (3.7)	81.4 (4.2)
MSNA incidence (bursts·100 heartbeats^{-1})								
SL saline	26 (18)	26 (19)	27 (19)	25 (20)	21 (16)	23 (12)	22 (13)	23 (11)
SL dopamine	29 (20)	32 (20)	33 (20)	30 (16)	27 (14)	27 (16)	35 (14)	31 (12)
ALT saline	37 (16)	35 (16)	32 (16)	33 (16)	36 (16)	33 (16)	34 (16)	34 (16)
ALT dopamine	40 (16)	35 (14)	40 (14)	42 (12)	37 (15)	38 (12)	38 (13)	35 (9)
HR (beats·min^{-1})								
SL saline	65 (10)	66 (10)	70 (11)	72 (11)	74 (11)	76 (13)	80 (10)	82 (12)
SL dopamine	69 (10)	71 (10)	73 (11)	75 (12)	76 (13)	78 (12)	83 (11)	87 (10)
ALT saline	74 (8)	76 (9)	77 (9)	80 (11)	84 (13)	87 (10)	90 (12)	95 (14)
ALT dopamine	77 (8)	83 (7)	84 (10)	84 (11)	86 (12)	90 (10)	95 (9)	99 (10)
SV (ml)								
SL saline	115 (12)	115 (11)	114 (13)	113 (13)	114 (14)	114 (13)	113 (13)	113 (13)
SL dopamine	120 (11)	121 (10)	120 (10)	120 (11)	118 (9)	120 (10)	118 (12)	118 (13)

ALT saline	113 (7)	111 (5)	112 (6)	110 (6)	110 (6)	112 (9)	115 (9)	116 (8)
ALT dopamine	117 (11)	116 (10)	114 (12)	114 (11)	114 (8)	114 (8)	113 (10)	114 (9)

$P_{ET}O_2$, partial pressure of end-tidal oxygen; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; MSNA, muscle sympathetic nerve activity; HR, heart rate; SV, stroke volume. Data expressed as mean (standard deviation).

Table 2. P values derived from repeated measures ANOVA in which the factors of altitude (SL vs. ALT), infusion (saline vs. dopamine) and incremental hypoxia test stage (stages 1-8) were considered, as well as all two- and three-way interactions.

	Altitude	Infusion	Stage	Altitude x Infusion	Altitude x Stage	Infusion x Stage	Altitude x Infusion x Stage
$P_{ET}O_2$	0.562	0.461	**0.000**	0.347	0.291	0.297	0.668
$P_{ET}CO_2$	**0.000**	**0.015**	**0.000**	0.141	**0.000**	0.479	0.260
SpO_2	0.331	0.626	**0.000**	0.729	0.243	0.629	0.617
V_E	**0.000**	**0.019**	**0.000**	**0.034**	**0.000**	0.085	0.072
V_T	**0.000**	**0.022**	**0.000**	0.184	**0.000**	0.343	0.236
R_f	**0.001**	0.089	**0.000**	0.061	**0.001**	0.657	**0.035**
MSNA frequency	**0.025**	**0.034**	**0.002**	0.961	0.236	0.065	0.109
MSNA incidence	0.174	0.081	0.375	0.106	0.353	0.133	0.092
MAP	**0.000**	0.372	**0.000**	0.371	**0.000**	0.227	0.128
CO	**0.033**	**0.010**	**0.000**	0.798	**0.036**	0.205	0.517
TPR	**0.030**	**0.046**	**0.000**	0.805	0.176	0.279	0.678
HR	**0.002**	0.101	**0.000**	0.840	0.218	0.449	0.712

SV	0.286	0.107	0.249	0.323	0.266	0.109	**0.026**

$P_{ET}O_2$, partial pressure of end-tidal oxygen; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; V_E, minute ventilation; V_T, tidal volume; R_f, respiratory frequency; MSNA, muscle sympathetic nerve activity; MAP, mean arterial pressure; CO, cardiac output; TPR, total peripheral resistance; HR, heart rate; SV, stroke volume.

Table 3. P values derived from repeated measures ANOVA in which the factors of infusion (saline vs. dopamine) and incremental hypoxia test stage (stages 1-8) and their two-way interaction were considered separately at SL and ALT.

	SL			ALT		
	Infusion	**Stage**	**Infusion x Stage**	**Infusion**	**Stage**	**Infusion x Stage**
$P_{ET}O_2$	0.809	**0.000**	0.310	0.376	**0.000**	0.457
$P_{ET}CO_2$	**0.025**	0.523	0.637	0.177	**0.000**	0.351
SpO_2	0.743	**0.000**	0.603	0.417	**0.000**	0.841
V_E	0.298	**0.000**	0.517	**0.023**	**0.000**	0.073
V_T	0.120	**0.001**	0.259	**0.047**	**0.000**	0.283
MSNA frequency	0.051	**0.028**	0.091	**0.015**	**0.023**	**0.042**
MSNA incidence	0.053	0.332	0.201	0.063	0.313	**0.034**
MAP	0.590	**0.011**	0.565	0.308	**0.000**	0.133
CO	**0.041**	**0.000**	0.650	0.135	**0.000**	0.206
TPR	0.228	**0.000**	0.113	0.175	**0.000**	0.473
HR	0.273	**0.000**	0.682	0.218	**0.000**	0.515

$P_{ET}O_2$, partial pressure of end-tidal oxygen; $P_{ET}CO_2$, partial pressure of end-tidal carbon dioxide; V_E, minute ventilation; V_T, tidal volume; R_f, respiratory frequency; MSNA, muscle sympathetic nerve activity; MAP, mean arterial pressure; CO, cardiac output; TPR, total peripheral resistance; HR, heart rate; SV, stroke volume.

<u>Figure legends</u>

<u>Figure 1.</u> **Intravenous infusion of dopamine did not significantly modify steady-state respiration at Zurich (SL, 432 m) and the Jungfraujoch research station (ALT, 3,454 m) while participants breathed the ambient air.** VE, minute ventilation; VT, tidal volume; Rf, respiratory frequency. Data expressed as individual values and means with (standard deviation). ANOVA P values are displayed.

<u>Figure 2.</u> **Original sympathetic neurograms obtained at sea level and high altitude with infusion of saline and dopamine.** In this individual, a low level of MSNA was present at sea level, but the recording site was verified with a pronounced MSNA response to a breath hold. Note the minimal response to dopamine, but pronounced sympathoexcitation at altitude.

<u>Figure 3.</u> **Cardiovascular variables at Zurich (SL) and the Jungfraujoch research station (ALT) during infusion of saline and dopamine with participants breathing ambient air.** MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; TPR, total peripheral resistance; HR, heart rate; SV, stroke volume. Data expressed as individual values and means with (standard deviation). ANOVA P values are displayed.

<u>Figure 4.</u> **Respiratory responses to acute incremental hypoxia at Zurich (SL) and Jungfraujoch research station (ALT) during infusion of saline or dopamine.** VE, minute ventilation; VT, tidal volume; Rf, respiratory frequency. Data expressed as individual values and means with (standard deviation). ANOVA P values are displayed.

<u>Figure 5.</u> **Original sympathetic neurograms obtained during the initial (1) and final (8) stages of the acute incremental hypoxia test at sea level and high altitude with infusion of saline and dopamine.** Note the modest increase in MSNA in response with either acute incremental hypoxia or dopamine, and the pronounced sympathoexcitatation at ALT.

<u>Figure 6.</u> **Cardiovascular responses to acute incremental hypoxia at Zurich (SL, 432 m) and Jungfraujoch research station (ALT) during infusion of saline or dopamine.** MAP, mean arterial pressure; MSNA, muscle sympathetic nerve activity; TPR, total peripheral resistance. Data expressed as individual values and means ± standard error.

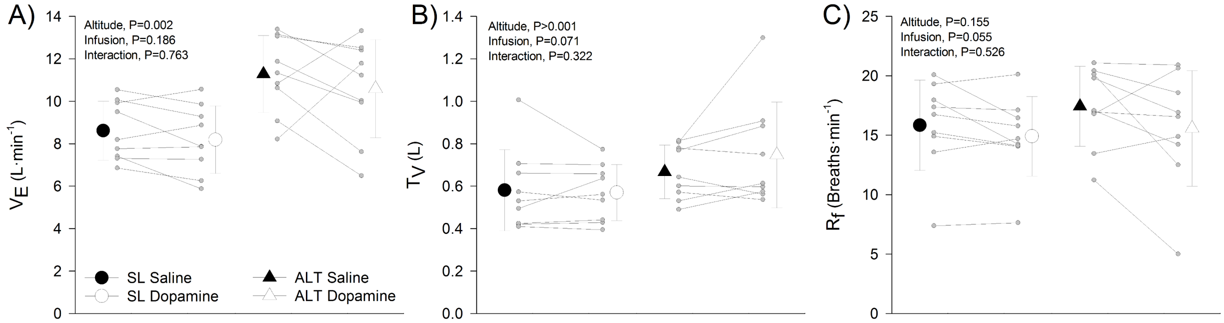

A)
Altitude, P=0.002
Infusion, P=0.186
Interaction, P=0.763
VE (L·min-1)
SL Saline
SL Dopamine
ALT Saline
ALT Dopamine
B)
Altitude, P>0.001
Infusion, P=0.071
Interaction, P=0.322
TV (L)
C)
Altitude, P=0.155
Infusion, P=0.055
Interaction, P=0.526
Rf (Breaths·min-1)

Figure 1

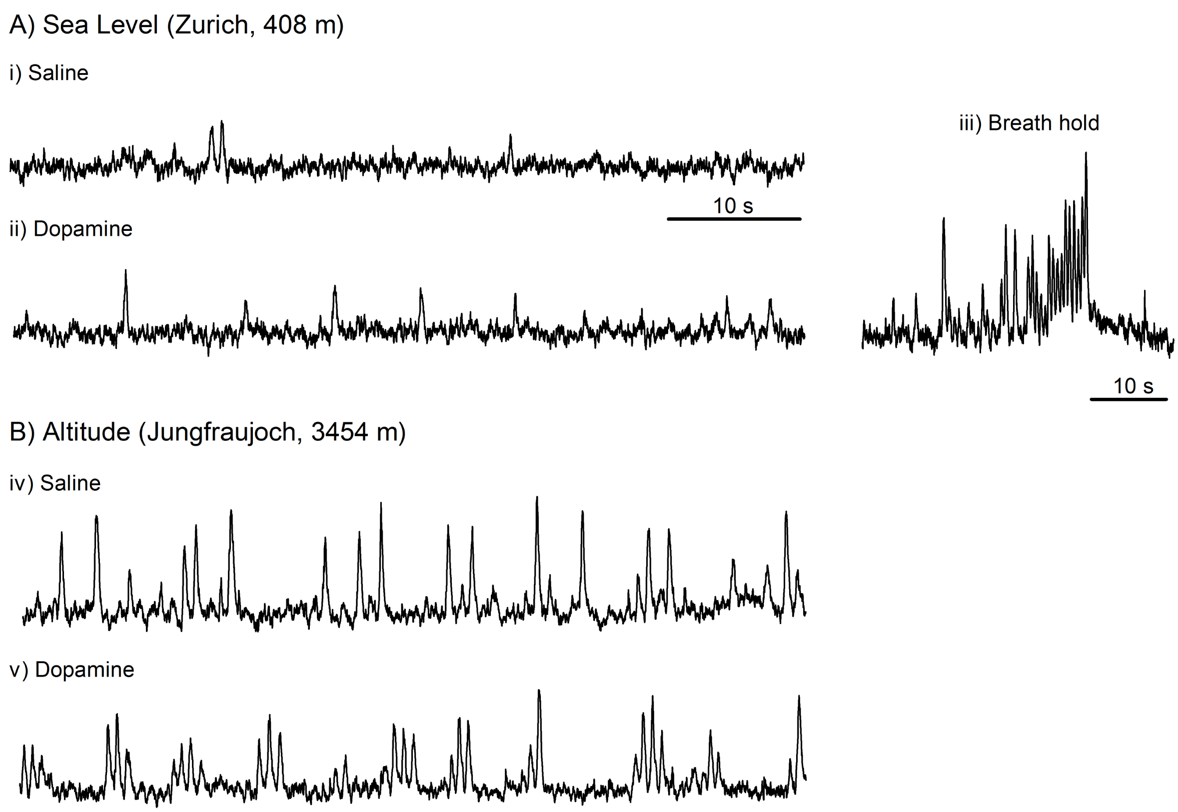
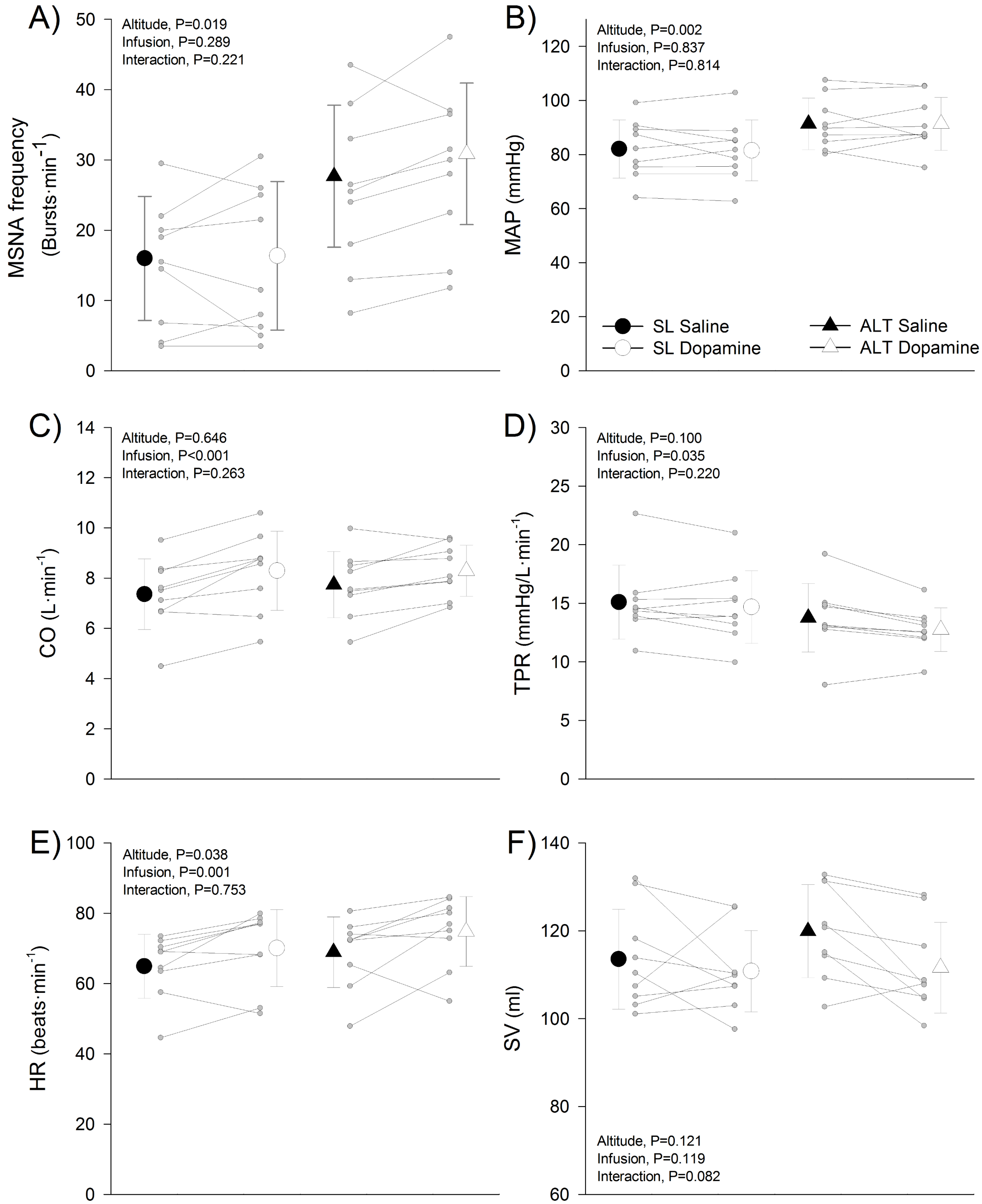

A) Sea Level (Zurich, 408 m)
i) Saline
iii) Breath hold
10 s
ii) Dopamine
10 s
B) Altitude (Jungfraujoch, 3454 m)
iv) Saline
v) Dopamine

Figure 2



A)
MSNA frequency (Bursts·min-1)
Altitude, P=0.019
Infusion, P=0.289
Interaction, P=0.221
B)
MAP (mmHg)
Altitude, P=0.002
Infusion, P=0.837
Interaction, P=0.814
SL Saline
SL Dopamine
ALT Saline
ALT Dopamine
C)
CO (L·min-1)
Altitude, P=0.646
Infusion, P<0.001
Interaction, P=0.263
D)
TPR (mmHg/L·min-1)
Altitude, P=0.100
Infusion, P=0.035
Interaction, P=0.220
E)
HR (beats·min-1)
Altitude, P=0.038
Infusion, P=0.001
Interaction, P=0.753
F)
SV (ml)
Altitude, P=0.121
Infusion, P=0.119
Interaction, P=0.082

Figure 3

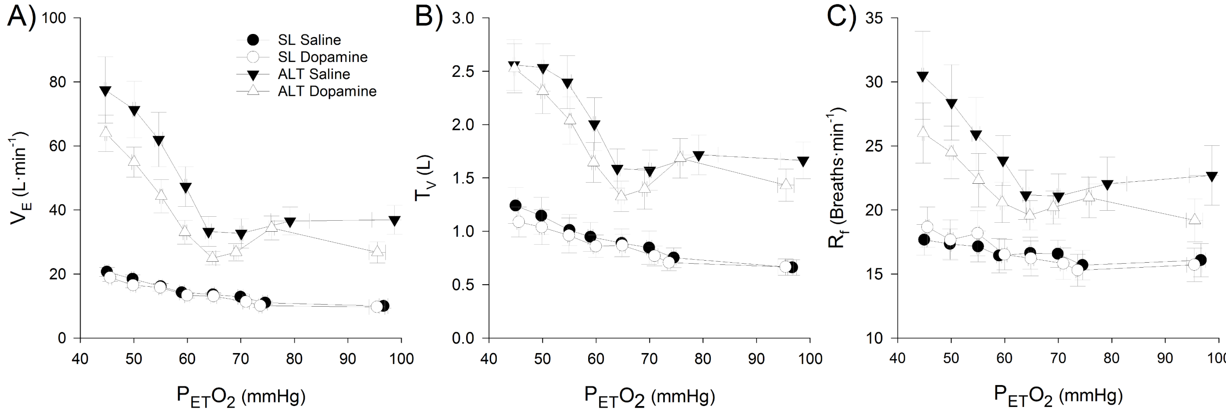
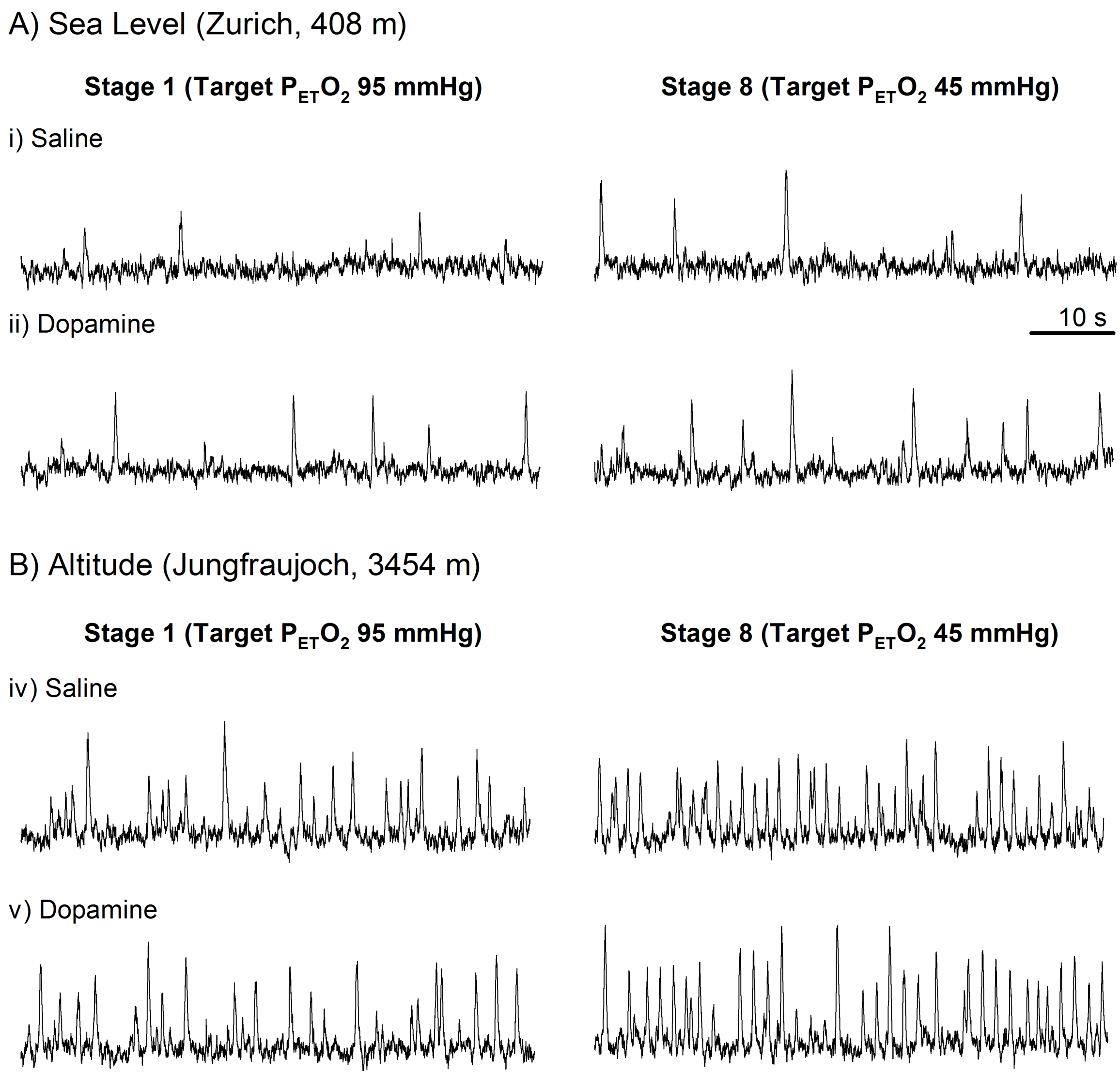
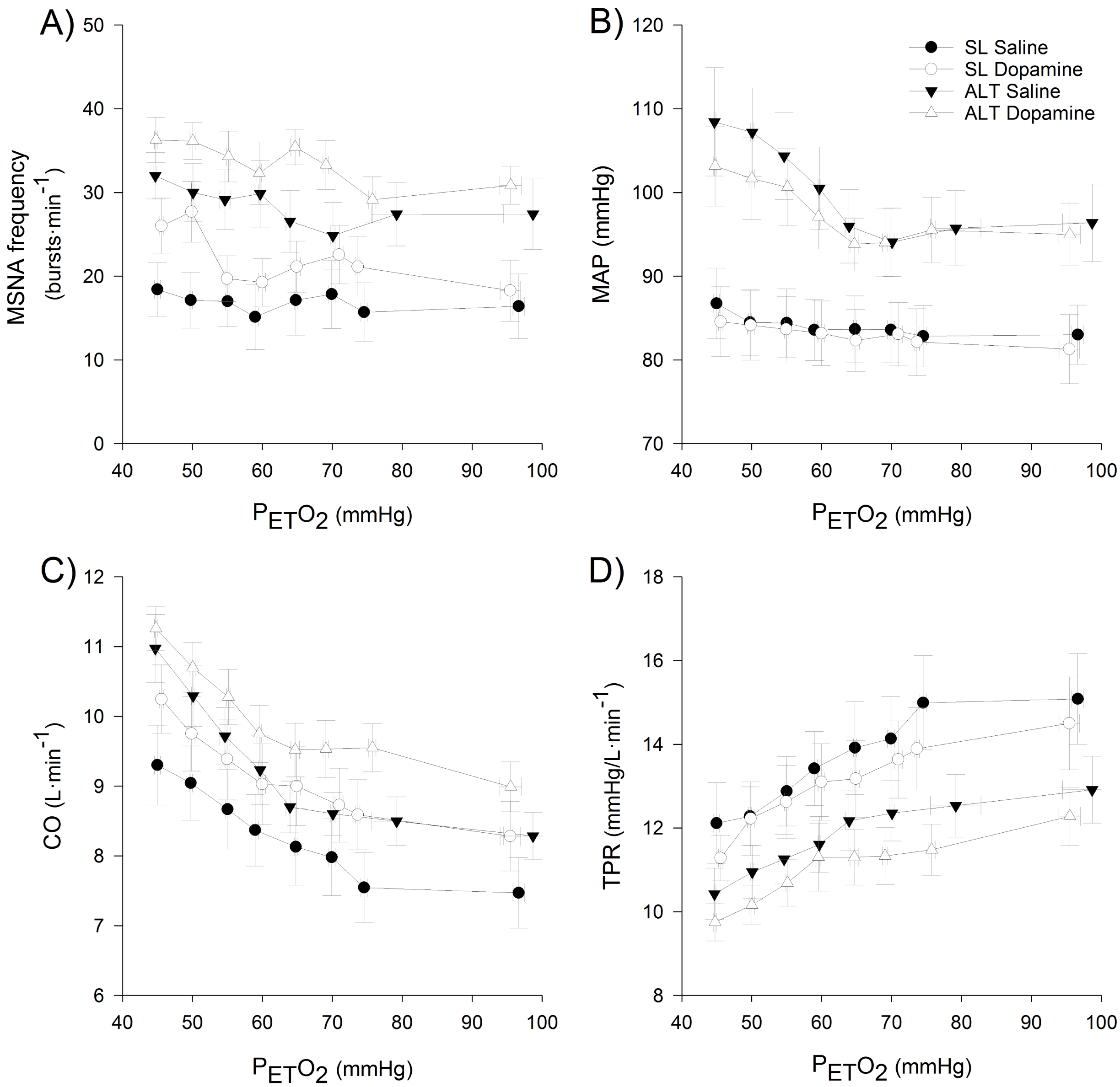

A)
B)
C)
SL Saline
SL Dopamine
ALT Saline
ALT Dopamine
V_E (L·min^-1)
T_V (L)
R_f (Breaths·min^-1)
P_ETO2 (mmHg)
P_ETO2 (mmHg)
P_ETO2 (mmHg)

Figure 4



A) Sea Level (Zurich, 408 m)
Stage 1 (Target $P_{ET}O_2$ 95 mmHg)
Stage 8 (Target $P_{ET}O_2$ 45 mmHg)
i) Saline
ii) Dopamine
10 s
B) Altitude (Jungfraujoch, 3454 m)
Stage 1 (Target $P_{ET}O_2$ 95 mmHg)
Stage 8 (Target $P_{ET}O_2$ 45 mmHg)
iv) Saline
v) Dopamine

Figure 5



A)
MSNA frequency (bursts·min-1)
B)
MAP (mmHg)
SL Saline
SL Dopamine
ALT Saline
ALT Dopamine
C)
CO (L·min-1)
D)
TPR (mmHg/L·min-1)
PETO2 (mmHg)

Figure 6

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